Solid crystal suspension of Efavirenz using hot melt extrusion: exploring the role of crystalline polyols in improving solubility and dissolution rate

Article (Accepted Version)

Solid crystal suspension of Efavirenz using hot melt extrusion: Exploring the role of crystalline polyols in improving solubility and dissolution rate.

Jaywant N Pawar[1], Ritesh A Fule[1,2], Mohammed Maniruzzaman[3], Dilip G Surywanshi[1], Santosh M Gejage[1], Purnima D Amin[1]

1. Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Nathalal Parekh Marg, Matunga, Mumbai-400019, Maharashtra, India.

2. Faculty of Pharmaceutics Department, H.K. College of Pharmacy, Relief Road, Oshiwara, Jogeshwari West, Mumbai-400102, Maharashtra, India.

3. Department of Pharmacy (Chemistry), School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QT, United Kingdom.

Correspondence

Mr. Jaywant N. Pawar
Senior Research Fellow
Department of Pharmaceutical Sciences and Technology,
Institute of Chemical Technology Elite Status,
Matunga, Mumbai – 400019, India.
Tel: +91-22- 3361 1111/2222, Fax: +91-22- 3361 1020
Email Id – jaywantpawar.ict@gmail.com / pd.amin@ictmumbai.edu.in

Solid crystal suspension of Efavirenz using hot melt extrusion: Exploring the role of crystalline polyols in improving solubility and dissolution rate.

Abstract:

The poor aqueous solubility of drugs has emerged as a major issue for pharmaceutical scientists from many decades. The current study explores the manufacture and development of a thermodynamically stabilized solid crystal suspension (SCS) of poorly water soluble drug efavirenz via hot melt extrusion. Efavirenz is a non-nucleoside reverse transcriptase inhibitor and belongs to BCS class II. The SCS was prepared using pearlitol and xylitol as a crystalline carrier. The drug-excipient blend was processed by hot melt extrusion with up to 50% (w/w) drug loading. Physico-chemical characterization of the SCS conducted via a scanning electron microscopy showed crystalline morphology. The solid state analysis undertaken by using differential scanning calorimetry and hot stage microscopy confirmed that SCS are in crystalline state. Similarly, X-ray powder diffraction analysis revealed that pure drug, crystalline carriers and developed SCS are in crystalline state. The FTIR chemical imaging analysis of SCS formulations showed a homogeneous drug distribution within respective crystalline carriers while an advanced chemical analysis via atomic force microscopy and Raman analysis complemented the foregoing findings of the FTIR imaging. The developed SCS1 formulation showed up to 81 fold increase in the solubility and 4.1 fold increase in the dissolution rate of the drug compared to that of the bulk substance. Surprisingly, the developed SCS formulation remained stable for a period of more than one year at accelerated conditions inferred from dissolution studies. It can be concluded that the SCS approach can be used as an alternative contemporary technique to enhance the dissolution rates of many other poorly water-soluble drugs by means of thermal HME processing.

Keywords: Efavirenz, xylitol, pearlitol, solid crystal suspensions, solubility, stability.

1. Introduction

In the recent years, hot-melt extrusion (HME) has been evolved as a promising pharmaceutical manufacturing technology for a wide variety of pharmaceutical applications such as taste masking of API (Maniruzzaman et al., 2014;), sustained oral drug delivery (Claeys et al., 2015;), cocrystal drug delivery (Moradiya et al., 2014). More recently HME has successgfully been used as a continuous manufacturing technique by implementing the Quality by design (QbD) and process analytical technology (PAT) paradigm (ref). Different types of

dies may be employed to obtain desired shape of extrudes via HME. Using these different types of dies formulation products such as oral fast dissolving films, transmucosal, transdermal DDs (Park et al., 2015), intravaginal DDs (Clark et al., 2012), implants (Stanković et al., 2013), stents and ophthalmic inserts (Lee et al., 2010) have been manufactured and reported in literature. There are several commercial products available in market to date primarily made by HME and these include Ritonavir - Norvir® (Sherman and Steinberg, 2011), Ibuprofen - Nurofen® (Gryczke et al., 2011), Lopinavir/Ritonavir - Kaletra® (Klein et al., 2007), Metformin HCL/Vildagliptin - Eucreas® (Stanković et al., 2015) and Greisofulvin Gris-PEG® (Stanković et al., 2015) etc.. The HME has successfully be optimized and exploited in solubilization of poorly soluble drugs in crystal engineering and by formation of pharmaceutical cocrystals (Dhumal et al., 2010; Moradiya et al., 2014)

Recently, a new phrase called solid crystal suspensions (SCS) was proposed, which is an intimate mixture of a crystalline drug with crystalline carrier resulting in the formation of a stable formulation with significantly faster dissolution rate, comparative to pure drug using HME (Reitz et al., 2013; Thommes et al., 2011). In SCS, the crystalline drug is suspended in crystalline carrier matrix without facilitating any interactions between the drug and the crystalline matrices but producing a thermodynamically stable system. Unlike the starting components, the newly formed crystallinelattice also completely differs from the traditional solid dispersions (Chiou and Riegelman, 1971; Marsac et al., 2006). The physical stability of SCS is very high because of no existence of amorphous phase in the system , compared to other types of traditional solid dispersions (Thommes et al., 2011; Urbanetz, 2006). The increase in dissolution rate by SCS approach is due to API particle size reduction within the highly soluble crystalline carrier and improvement in wettability and thus the solubility.

EFV is used as a part of highly active antiretroviral therapy (HAART) in the treatment of human immunodeficiency virus (HIV) type 1 disease. It is a white, crystalline, non-hygroscopic powder which is insoluble in water (Zakeri-Milani et al., 2009). Hence, there is strong need to overcome the issue of poor solubility of EFV in order to enhance oral bioavailability. It has been reported in literature that various polyols, superdisintegrants and polymers can be used as a carrier in HME for solubilization of poorly water-soluble drugs (Moradiya et al., 2015;).

The aim of the study was to explore SCS concept for poorly water-soluble drug EFV using highly soluble crystalline carriers by HME technology. In this context, we report the formation of crystalline molecular dispersions of poorly water-soluble drug Efavirenz (EFV)..

In the present study, we also have explored polyols such as xylitol and mannitol as a crystalline carrier for SCS formation, due to their rapid crystallization behaviour (Cares-Pacheco et al., 2014)as reported earlier (Thommes et al., 2011). The physico-chemical characterization of the SCS and the stability of the final optimized formulation was thoroughly accessed.

2. Material and methods:

2.1 Materials

Efavirenz was obtained as a gift sample from Laurus labs, India. Mannitol (Pearlitol 50 C) was obtained from Rouquette, France and Xylitol from Signet chemicals, India. All the solvents used were of analytical grade obtained from Sigma Aldrich chemicals, India.

2.2 Selection of polyols as carrier for HME:

The solubility parameter difference ($\Delta\delta$) between API and excipient gives possible idea on the miscibility of the drug delivery system (Van Krevelen and Te Nijenhuis, 2009). If the solubility parameter difference is less than 7 $MPa^{1/2}$ difference is generally accepted as an indication of miscibility whereas for a difference of more than 10 $MPa^{1/2}$ the system is likely to be immiscible (Greenhalgh et al., 1999; Dukeck et al., 2013). Efavirenz is poorly water-soluble drug (M.P. 138-140°C), which is highly hydrophobic. Hence, its solubility is sturdily hindered by high crystal lattice energy. Thus, we choose polyols such as xylitol and pearlitol as hydrophilic crystalline carriers for solubility enhancement of EFV. Solubility parameters usually used to access mixing capability between polymers and drugs as reported in literature (Fule et al., 2016; Lu et al., 2015). Based on the group contribution method calculation solubility parameters of EFV, pearlitol (mannitol) and xylitol are 24.55 $MPa^{1/2}$ (Sathigari et al., 2012), 40.5 $MPa^{1/2}$ (Thommes et al., 2011), and 36.86 $MPa^{1/2}$ (Kitak et al., 2015), respectively. Solubility parameter difference ($\Delta\delta$) between EFV and carriers is between 11.5-15 $MPa^{1/2}$. We have purposely chosen carriers that have a solubility difference within the limit for immiscibility in order to articulate the effect of the critical parameters like drug-polymer ratio, screw speed, extrusion temperature, residence time and stability of final dosage formulation.

2.3 Preparation of EFV solid crystal suspensions by HME

Hot melt extrusion was carried out using a Thermo Scientific HAAKE™ MiniLab II Micro compounder (Thermo scientific, Newington, NH, USA) with co-rotating twin screws. Batches were taken in different ratio of EFV to carriers to optimize the final formulation.

Optimized batches were taken in the ratio of EFV to either xylitol or pearlitol by preparing physical mixture using mortar-pastle. HME was performed having drug to carriers in 20:80 and 50:50 ratio of both the polyols as shown in table 2.

Accurately 20 gm powder physical mixtures were prepared and extruded through the 1 mm diameter die at optimized temperature with 100 rpm speed (Chen et al., 2006). For melt extrusion with pearlitol, the extrusion was carried out at temperature 138-140°C, (melting point of EFV), with the screw speed of 100 RPM. Both xylitol and pearlitol crystallizes after the melt extrusion process, the extrudates obtained were further cooled at room temperature and grinded to form powdered material and termed solid crystal suspension. SCS samples were kept in umber colored bottles for further physicochemical characterization.

2.4 Thermogravimetric analysis (TGA)

TGA studies were carried out using Mettler Toledo TGA/SDTA (Mettler Toledo, Switzerland) operating with Stare software version Solaris 2.5.1. Accurately weighed (3–5 mg) sample was loaded in alumina crucible and heated at the rate of 10 °C/min over a temperature range of 35–250 °C, under nitrogen purge (50 mL/min), to determine loss in weight.

2.5 Saturation solubility study

The EFV and SCS were analysed for saturation solubility study in 0.1N HCl containing 0.2% sodium lauryl sulfate (SLS) maintained at 37 ± 0.5 °C. Excess amounts of API and SCS were added to 10 ml of dissolution media and capped glass test tubes were kept in a shaking incubator (Boekel scientific, USA) maintained at 37±0.5°C with75 rpm speed for 48 hrs. The solutions in the test tubes were vortexed and kept for centrifugation at 5000 rpm for 10 min. The supernatant layer was then filtered through 0.45µm millipore membrane filter and analyzed for drug content by HPLC. The study was carried out in triplicate.

2.6 *In vitro* dissolution studies

The *In vitro* dissolution studies were conducted using a USP type II dissolution apparatus. From SCS amount equivalent to 100 mg of EFV was filled into capsules and the capsules were then placed in sinker and put into dissolution medium within the apparatus. The dissolution studies were performed under sink conditions and the medium was 1000 mL of 0.1N HCl containing 0.2% sodium lauryl sulfate (SLS) maintained at 37 ± 0.5 °C with speed 50 rpm. At various time points like 15, 30, 45, 60 and 90min, the samples were withdrawn and an equal amount of fresh medium was added to the ongoing dissolution medium vessel. These

samples were analysed using UV-spectrophotometer at 248 nm. The dissolution studies were performed in triplicate.

2.7 Differential Scanning Calorimetry (DSC)

DSC analysis was performed to check the physical state of SCS with respect to pure EFV, SCS, xylitol and pearlitol using Pyris-6 DSC Perkin Elmer (Lee et al.). Approximately 3-4 mg of sample was hermetically sealed in aluminum pan. An empty aluminum pan was used as a blank. Samples were heated at the rate of 10°C/min^{-1} from 30°C - 300°C under an inert atmosphere was maintained purging nitrogen gas at a flow rate of 18 ml/min. The Pyris manager® software was used for post experimental analysis.

2.8 Hot stage microscopy (HSM)

HSM was carried out to observe thermal transitions in the SCS formulations. Leica 160DMLP polarized microscope (Leica Microsystems Wetzlar GmbH, Wetzlar, Germany) equipped with Linkam LTS hot stage was used for this study. SCS samples was mounted in oil on glass slide and heated from ambient temperature to 200 °C, at a heating rate of 10 °C/min. Changes in morphology behaviour in all samples were collected as a video recording using JVS color video camera and analyzed using Linksys32 software. This is potential technique to understand the morphological behavior of extruded material.

2.9 X-ray diffraction (XRD) analysis

XRD was used to determine the physical state drug in SCS as compared to pure carrier materials. X-ray diffraction using an X-ray diffractometer (Bruker D8 Advance, WI, USA) with a scan speed of 2°/min over a range of 2–50 (2Θ) was used. The samples were placed in a zero background sample holder and incorporated on a spinner stage.

2.10 Structural analysis by FTIR

FTIR spectra of the EFV and SCS were recorded using a Fourier transform infrared spectrophotometer model 4100 (Spectrum GX-FT-IR, Perkin Elmer, USA) to investigate any possible interactions between the drug and carriers. The samples were premixed with KBr using mortar and pestle and KBr disks were prepared using hydraulic press. The scanning range was set at 4000 to 400 cm^{-1} with resolution of 4 cm^{-1}

2.12 FTIR spectroscopic Imaging

The optimized SCS formulation was characterized for FTIR imaging. Vertex 80/ Hyperion 3000, Bruker, Germany) instruments with liquid nitrogen cooled single mercury-cadmium-telluride (MCT) focal plane array detector 128 × 128, (Santa Barbara Focal plane, Goleta, California) at range: 4000-900 cm^{-1} was used for the analysis(Verma et al., 2012). Then The powder sample was placed on temperature-controlled stage. Position of the accessories were adjusted such that a good focused image was obtained. The spectrometer was setup in attenuated total reflectance (ATR) mode using a diamond internal reflection element (IRE). The images were acquired with a spectral resolution of FTIR 0.2 cm^{-1} and 32 co-added scans by using OPUS® 6.5 software with an acquisition time of approximately 2 min.

2.13 Raman spectra and mapping

The Raman spectra of the SCS formulations were recorded with a LabRamHR800 (Horiba Jovan Yvon, UK) equipped with a 633-nm Ar–Ne laser. The laser excitation light was transmitted through a notch filter towards confocal hole, and entrance slit of spectrograph. The stokes-shifted Raman scatter was dispersed using 1800 groove/min grating onto a peltier-cooled changed-coupled device (CCD, Andor Technology PLC) to capture a spectrum. Each samples spectra measured using continuous readout of Raman shift spectrum from 2800 to 3000 cm^{-1}. In all experiments, a near-IR laser (785 nm) of 250 mW power was employed. Spectra were acquired using a 50 × objective and a 300 µm confocal hole. Raman spectras were collected using a SYNAPSE CCD detector (1024 pixels). For milled extrudate compact, maps were acquired over 8-10 min 2800 to 3000 cm^{-1} regions using a fixed grating to allow relatively rapid mapping. Raman mapping or imaging of SCS was carried out to understand the drug distribution pattern inside polymer matrix and check its uniformity (Widjaja et al., 2011). Analysis of data sets can be performed quickly and simply to provide information on peak parameters (e.g., position, width, area). LabSpec 6 software is used to run the analysis (Fule et al., 2016; Furuyama et al., 2008).

2.14 Preparation of extrudates for AFM characterization

Fractured fresh extrudate with smooth surfaces were used for microscopic investigations using AFM. The freshly fractured extrudates were mounted on an optical glass slide by use of a 2 component epoxy resin, which hardened within ~6 min to get fracture surface as horizontal as possible, so that it shall facilitate to enable non-destructive imaging (Turner et al., 2007). JXA-8530F Hyper Probe Electron Probe Micro-analyzer instrument by JEOL was employed for AFM analyses. Freshly fractured extrudates on microscopic glass slides were

mounted on the micrometer positioning stage of a dimension icon AFM with accelerating voltage of 1-30kV. Probe current range was kept between 10 pA to 200 pA and images were obtained. An atomic force microscope (AFM) scans the surface of a specimen with a very sharp tip mounted to a cantilever, deflections of which are directly related to surface nanoscale topography and various other physical properties. Between 10 and 25 regions per sample were programmed to be automatically characterized using the software routine "programmed move" in tapping Mode. Height, phase, and amplitude images were collected simultaneously, using etched silicon cantilevers with a nominal spring constant of k=40–100 N/m (JEOL AFM Probes). Image areas of 10×10 mm were recorded at a resolution of 1024×1024 pixels. All data were batch-processed using Scanning Probe Image Processor (SPIP 5.1.1) (Fule et al., 2016; Lauer et al., 2013).

2.15 Analytical method (HPLC)

The assay of EFV SCS was assessed using high-performance liquid chromatography (HPLC) system of JASCO corporation equipped with auto sampler (AS-2055 plus, intelligent sampler), photodiode array detector (JASCO corp.). A Phenomenex Luna® reverse-phase C18 column (150 x 4.6 mm; 5µm particles) was used as a stationary phase. The mobile phase was composed of a mixture of buffer (Ammonium acetate buffer, pH maintained at 7.5): acetonitrile in the ratio 40:60 (v/v). The buffer was prepared by dissolving ammonium acetate in 1000 mL of water; maintain the pH at 7.5 ± 0.05. The flow rate was 1.5 mL/min, with injection, volume was 20 µL and the detection of EFV was done at 248 nm with the retention time of 3.38 ± 0.05min. Drug content uniformity was assessed by accurately weighing SCS equivalent to 10 mg of EFV were dissolved in 10 mL of methanol and appropriately diluted. These samples further centrifuged (Centrifuge Eppendorf) for 5 min at 5000 rpm and drug content was quantified using previously delineated HPLC procedure.

2.16 Stability studies

The impact of temperature and humidity conditions on chemical and physical stability of the EFV SCS was determined according to ICH guidelines. A portion of EFV SCS filled into capsules was stored in a HDPE bottles to at 40°C/75% relative humidity for 12 months. Differential scanning calorimetry and powder XRD studies were carried out to determine crystallinity of EFV SCS at 0, 6 and 12 months. The *in vitro* dissolution studies also carried out to study the percentage drug release of aged SCS samples.

3. Results and discussions:

3.1 TGA analysis

In case of HME processing, the study of thermal profiles of API and individual excipients employed in a formulation is considered a decisive step. The EFV and individual polyols used in the formulations were evaluated for thermal stability at elevated temperatures. TGA studies revealed that EFV, xylitol and pearlitol were stable under the employed extrusion conditions and exhibited less than 2% weight loss, when subjected to heat ramp (Fig.1). In this context, all formulations were processed below 150°C temperature, henceforth it was concluded that processing temperatures studied were suitable during the HME process.

3.2 Saturation solubility study

The solubility of SCS was found to be improved in both dissolution media distilled water (1) and 0.1N HCl (2) containing 0.2% SLS (DiNunzio et al.). The apparent solubility of pure EFV was found to be 9 ± 1.12 µg/ml and 0.212 ± 0.32 mg/mL in dissolution medium 1 and 2, respectively. The solubility of SCS was substantially improved as shown in Fig 2. The apparent solubility of SCS was maximum for SCS1 formulation with about 161 µg/ml and 1.67 mg/ml solubility in dissolution medium of 1 and 2, respectively. It is about 19 and 80 folds increase in the solubility compared to that ofof pure EFV. The solubility of SCS is improved due to the highly hydrophilic nature of pearlitol and xylitol in water. It has been reported in literature that polyols facilitates hydration of hydrophobic regions in drug molecules (Arakawa and Timasheff, 1982). The same kind of effect would result in to favored wetting of hydrophobic drug particles. Xylitol and pearlitol acts as a better solvent carrier in HME process facilitating improved solubility by imparting wetting to drug particles (Thommes et al., 2011).

The SCS formulation approach developed by HME process, results in the formation of a suspension of API in molten carrier system of polyols. The molten xylitol found somewhat better solvent for EFV due to its relatively lowermelting point (94-96 °C) and acts as a better carrier to make a molten mass with EFV resulting in formation of solid crystal suspensions. The improved solubility of EFV SCS results due to improved wettability and reduced particle size. The extrusion process results in to reduction in particle size of drug (Reitz et al., 2013).

Remarkably, SCS2 and SCS4 formulations reveals smaller particle size than that of with SCS1 and SCS3. The smaller particle size of SCS2 and SCS4 is ascribed to lower shear forces in 20% drug loaded formulations, which exhibits, having availability of higher proportion of molten xylitol and pearlitol in formulation mixture during hot melt extrusion processing (Thommes et al., 2011). In extrusion process, the intense mixing and agitation of formulation system results in uniform distribution of fine particles.

The improved solubility of solid crystal suspension formulations imparts due to physical configuration of hot melt extrudate than that of physical interaction between drug and polyols as crystalline carriers. The API particles become intimately mixed and entirely surrounded by highly hydrophilic carriers, resulting into improved wetting by aqueous dissolution medium due to increased surface area of drug particles leading to improved solubility of EFV SCS compared to pure EFV.

3.3 *In vitro* dissolution rate study

The *invitro* dissolution studies were conducted to assess the performance of SCS formulations compared to pure EFV. The Fig. 3 indicates dissolution profiles of pure EFV, SCS formulations and marketed capsule EFAVIR®. It has beenreported in literature that the bulk EFV has an aqueous solubility of 5.2 ± 0.3 µg/mL (Maurin et al., 2002). It can be seen that pure EFV showed slow dissolution rates of 22% at 45 min, 24% at 90 min. In contrast, all SCS formulations showed significant increase in dissolution rate of more than about 70% at the end of one hour. Optimized batch i.e. SCS1 (20% EFV) and SCS2 (50% EFV) formulated with xylitol showed the dissolution of 100% and 93%, respectively in 1hr, which is approximately 4.1 and 3.9 folds higher than that of pure EFV. In addition, the SCS3 (20% EVF) and SCS4 (50% EVF) made using pearlitol showed slightly slower dissolution of 88% and 82%, respectively after 1 hr. In all formulations, dissolution rate has been significantly improved compared to that of pure EFV ($P < 0.05$). The corresponding physical mixture of EFV with xylitol and pearlitol showed little increase in dissolution rate. However, dissolution from physical mixture is not as effective as that from the developed SCS formulation. The marketed EFAVIR® capsule showed 98% drug release in 1 hr, which is similar to that of the optimized SCS formulation ($P < 0.05$ Fig.3).

It is expected that the solid crystal suspensions prepared of crystalline materials will enhance the physical and chemical stability of the drug. Therefore, the stability studies conducted at accelerated condition for 6 months showed no significant change in the release of the crystalline poorly soluble EVF.The Fig.4 shows dissolution profiles of SCS1 and SCS3

formulations kept for accelerated stability conditions (40° C/ 75% RH) at day 1, 6 months and 12 months. Differences in dissolution profiles between fresh and 12 month's stored samples are statistically non-significant ($P< 0.05$). The formulations SCS3 and SCS4 produced using pearlitol can be considered physically stable. In contrast, SCS1 showed statically significant difference ($P < 0.2$) in dissolution profiles during the storage at the accelerated condition. The SCS prepared from xylitol showed physical and chemical instability and showed decrease in dissolution rate (Rowe et al., 2009). This could be attributed to the nature of the possible interaction between the drug and the crystalline excipients during the extrusion.

3.4 Differential Scanning Calorimetry (DSC)

The thermal transitions of the bulk EFV, excipients and the extruded formulations were studied by DSC and are depicted in Fig. 5. EFV (Fig. 5.a) was characterized by thermogram showing a single, sharp melting endothermic peak at 139.60°C indicating crystalline nature of drug. The xylitol (Fig 5b) showed a sharp endotherm at 92.8°C corresponding to its melting and confirming its crystalline nature. Pearlitol showed a sharp endotherm peak 164.8°C inferring to its crystalline nature. In case of all SCS formulations, two melting endotherms have been seen (Fig 5c, d, f,g). The melting endotherm of xylitol and pearlitol were found broadened with less intensity and were shifted to slightly lower temperature compared to the bulk substances in all SCS formulations. This was expected since the presence of a molten liquid of sugar alcohols often reduces, up to certain extent, than melting point of the higher melting component. There were no glass transitions observed for either the excipients or final SCS extrudates, probably due to the strong crystallization tendency of both xylitol and pearlitol (Yu et al., 1998; Zhang et al., 2012).

The SCS appeared to be a physical mixture of two components in different proportions for both polyols systems. In case of SCS granulate melting peaks were found broader for xylitol and pearlitol, this may be due to heat flux in respective sample and is artifact of DSC analysis (Reitz et al., 2013).

3.5 Hot stage microscopy:

HSM studies were conducted to visually determine the thermal transitions and extent of melting of EFV, SCS1 and SCS3 extrudates prepared by HME. Fig. 6A (EFV), Fig. 6B (SCS1) and Fig. 6C (SCS3) infers the melting pattern at different stages such as notation 1 infers initial melting, 2 – onset of melting and 3 – infers complete melting of the respective extrudate and pure EFV and SCS formulations at different points as shown in Table 3.

The hot stage microscopy results are in good agreement with DSC results as the complete melting of respective substances was as same as DSC endotherm peaks. HSM technique has advantage as this microscopic method can be applied to more mixture systems compared to other analytical methods due to fewer assumptions involved (Yang et al., 2011). The marked temperature at left bottom corner of each screen indicates temperature of sample (A1-intinal melting, A2-onset of melting, A3-complete melting). The desolvation of respective materials was confirmed by various events in HSM analysis. EFV shows needle shaped crystals, which were transparent at room temperature. At respective onset of melting point showed crystals quite deformed and opaque in temperature range 135-145° C, confirming melting of EFVas indicated by DSC and TGA.

3.6 X-ray diffraction spectroscopic analysis:

The XRD diffratcograms of EFV, xylitol, pearlitol and respective SCS are shown in Fig. 7. The PXRD diffractogram of EFV shows sharp multiple peaks, signifying the crystalline nature of drug. The xylitol, pearlitol also show sharp intensity peaks due ot its crystalline structure.,Similarly, all of theextruded formulations show characteristic intensity peaks due to the presence of the crystalline substances in the formulations. The XRD diffraction peaks of SCS granulate infer that the obtained SCS formulation is a physical mixture of two compounds (Reitz et al., 2013).

During the extrusion process, pearlitol (i.e. mannitol) which is occurred in its β form, converted into more stable form of β– modification of mannitol instead of converting to its metastable α formas reported in previously by Thommes et. al (Thommes et al., 2011). This was attributed to the partial melting of the mannitol particles during the HME process. However, the existence of β –transformation seeds in the melt extrudates favors crystallization of the stable β –modification, which is kinetically stable and remains as such for indefinite periods without spontaneous transformation (Reitz et al., 2013). This indicates that both the drug and mannitol are present in crystalline form in final extrudate as form β -mannitol.

In case of xylitol, all extruded formulations exhibited similar characteristic peaks due to the presence of the crystalline drug and the sugar (Fig. 8). Fast crystallization of the excipients having high melting points is a critical element for producing crystal suspensions. Xylitol having melting point of 96 - 97° C exhibited better carrier system than that of pearlitol with melting point of 165° C, where the drug was melted and adsorbed in the carrier system. The SCS3 and SCS4 showed sharp diffractogram peaks resembling the EFV, which was present in higher EFV loads.

399

400 3.7 FTIR analysis:

401 The FTIR spectra's of EFV, xylitol, pearlitol and SCS system are depicted in Fig. 9.
402 The FTIR spectra of EFV Fig. (9a) showed characteristics peak of 3314 cm^{-1} (-NH stretch),
403 1742 cm^{-1} (C=O stretch), 1492 cm^{-1} (C ≡ C benzene ring stretch), 1240 cm^{-1}(-CN stretch), 1165
404 cm^{-1} (CO stretch), 1096, 1057 and 1074 (C-O-C stretch) and 689 and 652 cm^{-1} (-CF stretch).
405 Xylitol Fig. (9b) as well as pearlitol Fig. (10e) showed characteristics broad peak at 3100 –
406 3400 cm^{-1} resembling the maximum number of hydroxyl moieties (-OH stretch). In addition to
407 that 1165 cm^{-1} (-CO stretch), 1050 – 1300 cm^{-1} confirms the carboxylic acid and alcohol groups
408 moieties. The FTIR spectra's of the developed SCS1 and SCS2 formulations are shown in Fig.
409 (9c, d) and SCS3 and SCS4 formulationsin Fig. (10 f, g) which infers the combination of
410 characteristics peak of EFV and respective polyols. These results resemblance that formation
411 of SCS system by HME did not result in any molecular interaction between EFV and respective
412 carriers (Thommes et al., 2011). The characteristics broad peak stretch at 3100 – 3400 cm^{-1}
413 (shown by dotted rectangle) was retainedin the SCS system inferring to maximum number of
414 hydrophilic groups, which has been provided by hydrophilic carriers (Pawar et al., 2015). The
415 wave stretch of 2243 cm^{-1} corresponding to the –CN stretch of EFV was distinctively seen in
416 all SCS1, SCS2, SCS3 and SCS4 formulations (shown from top side by arrow). Henceforth the
417 SCS formulations may result in diffusion into dissolution medium and accelerated release of
418 the developed SCS formulations compared to pure EFV.

419

420 3.8 FTIR chemical imaging analysis

421 FTIR chemical imaging results of SCS3 has been shown in Fig. 11. The 3D image and
422 IR reflectance spectrum of point of interest of SCS3 specifies the surface compositional
423 homogeneity of EFV in pearlitol matrices. Use of FTIR imaging spectrum to understand
424 distribution of API at microscale level been reported in literature previously (Alhijjaj et al.,
425 2015; Ewing et al., 2015; Feng et al., 2015; Vo et al., 2016).

426 FTIR imaging was constructed based on ATR mode using a diamond internal reflection
427 element (Irene et al.). To understand the homogeneity of EFV in SCS3 at microscale level, the
428 extrudate was ground to powder pror to the imagingRandom spots on the extrudates were
429 analysed by ATR. The wave number of 2243 cm^{-1} corresponding to the –CN stretch, uniquely
430 distinguishes in EFV in the SCS3 (Fig. 11C) was used to generate the FTIR chemical images
431 (Pawar et al., 2016a). The peak range at 2186 cm^{-1} to 2289 cm^{-1} was selected as the chemical
432 imaging reflectance spectrum for both analysis. Fig. 11A and B represents the 3D image and

C shows the reflectance spectrum (Vo et al., 2016). The 3D graph is illustrated by distribution of the EFV by red colors and pearlitol as carrier system by green-yellow color. From this FTIR chemical imaging analysis study, it can be concluded that EFV is distributed in carrier systems homogenously. The results obtained by FTIR imaging could be a versatile analytical technique for characterization of extruded components for the distribution of API in polymer system by HME process (Vo et al., 2016).

3.9 Raman analysis

The intensity of spectral features in any particular solution is dependent on the concentration of particular species. The Raman spectra of SCS1 and SCS3 has been shown in Fig. 12. Raman spectroscopy and its mapping technique are useful tools to study the crystalline and amorphous states (Saerens et al., 2011), including discrimination of crystalline drug in SCS as shown in Fig**.** 12. Raman spectra are generally robust to temperature changes. In addition, by describing the distribution of the drug and respective carrier, it could be predicted how drug crystals formed during preparation. It also identified distribution of crystalline form of the drug substance and respective carrier. The presence of drug in crystalline form with uniform distribution in SCS in the images of the hydrophilic regions in SCS described by the width at around 3027 to 3091 cm^{-1} green area was thought to be the carrier. (Mishra et al., 2012). Green fluorescence is due to polymer matrix and blue related to the drug particles. The distribution pattern has been illustrated and the optical image confirmed the presence of drug distributed inside polymer matrix. (Fule et al., 2016; Saerens et al., 2011).

3.10 AFM analysis

Molecular fracture roughness data as displayed in 3D surface image and surface roughness data calculated from at least 10 images on each sample, which gives morphological surface interactions in detail of both SCS1 and SCS3 samples as shown in Fig.13. It can be claimed from the AFM analysis that there is a high level of surface interaction between the drug inside respective sugar matrix observed in extrudates. AFM characterization have the potential to identify the uniform molecular disperse mixture. AFM has potential to quantify different phases and it can be determined through imaging at molecular length scales, which results in short observation time (Fule et al., 2016). In current studies, the molecular interaction between the EFV and xylitol and pearlitol respectively has been quantified by AFM. Cross sectional view and 3D surface morphology of SCS shows molecular level of interactions between drug and polymer (Lauer et al., 2013). AFM probes were employed to characterize

and map the distribution of components within a SCSs prepared by HME. The distribution of drug inside polymer shows homogenous morphology (Pawar et al., 2016a). The two phases were mixed at molecular level as reflected by normal AFM image (nanoscale) and 3D image processed via AFM. These studies were performed on the stability samples (6 month old) which consequently confirms the stability of prepared SCS formulations.

3.11 Stability evaluation

Drug content of developed SCS extrudates was determined using HPLC analysis. EFV analytical method validation was carried out according earlier reported literature (Pawar et al., 2016b) Each batch of SCS were found in acceptable range as per US Pharmacopoeia for EFV. In addition, drug content analysis was also carried out for samples of 6 and 12-month accelerated storage conditions Fig. 14. It was found that drug content of SCS formulations made with pearlitol was in range of 96.03 to 101.60% with standard deviation values from 0.966 to 1.68, respectively for fresh and 12 months accelerated storage conditions samples. However, SCS made using xylitol as a crystalline carrier showed decrease in drug content from 100% to 87% and 99% to 91% in SCS1 and SCS2, respectively. The underlying reason may be thexylitol undergoing a caramelization reaction, and the tendency to form cakes upon storage to accelerated conditions (Repka et al., 2008; Rowe et al., 2009). Hence, SCS formulations made using xylitol failed in stability study. A further in detail study is needed to carry out to understand the instability issue. The results of the SCS formulations made using pearlitol as a carrier exibitshigh physical stability as well as chemical stability, confirmed from content uniformity values.

4. Conclusions

HME has successfully been exploited to manufacture EVF based SCS as an effective technique to increase the dissolution rate of poorly water soluble drug. In this emerging approach crystalline drug substance is keept in its crystalline state. The use of mannitol and xylitol as crystalline carriers showed a faster drug release compared to that of the bulk drug . The optimized SCS1 formulation system showed 19-81 fold improvement in the solubiltiy two different dissolution media and likewise the dissolution rates. The DSC and XRD analysis confirms that drug is present in stabilized crystalline form in the developed SCS formulation. FTIR analysis showed no chemical interaction between the API and sugar alcohols respectively. FTIR chemical imaging analysis has also confirmed the homogenous dispersions of the drug molecule into the sugar matrices. It can be concluded that the“solid crystal

suspension" approach by means of HME can be adopted as an alternative emerging technique particularly suitable for those drugs that are difficult to stabilize in amorphous form.

Acknowledgments

The author is thankful to University grants commission India for providing the research fellowship no. UGC-SAP/ICT-DPST/2012-13/1115. The authors want to acknowledge Laurus Labs, Hyderabad, India for genuine gift sample of Efavirenz. The authors are thankful to S.A.I.F., Department, Indian Institute of Technology, Mumbai for Raman analysis, AFM analysis and FTIR imaging analysis and interpretation.

References:

Alhijjaj, M., Bouman, J., Wellner, N., Belton, P., Qi, S., 2015. Creating drug solubilisation compartments via phase separation in multi-component buccal patches prepared by direct hot melt extrusion-injection moulding. Mol Pharm.2015

Arakawa, T., Timasheff, S.N., 1982. Stabilization of protein structure by sugars. Biochemistry 21, 6536-6544.

Cares-Pacheco, M., Vaca-Medina, G., Calvet, R., Espitalier, F., Letourneau, J.J., Rouilly, A., Rodier, E., 2014. Physicochemical characterization of D-mannitol polymorphs: the challenging surface energy determination by inverse gas chromatography in the infinite dilution region. Int J Ppharm 475, 69-81.

Chen, Z., Fang, P., Wang, H., Zhang, S., Wang, S., 2006. Property of ethylene vinyl acetate copolymer in melting processing. J Appl Pol Sci 101, 2022-2026.

Chiou, W.L., Riegelman, S., 1971. Pharmaceutical applications of solid dispersion systems. J Pharm Sci 60, 1281-1302.

Claeys, B., Vervaeck, A., Hillewaere, X.K., Possemiers, S., Hansen, L., De Beer, T., Remon, J.P., Vervaet, C., 2015. Thermoplastic polyurethanes for the manufacturing of highly dosed oral sustained release matrices via hot melt extrusion and injection molding. Eur J Pharm Biopharm 90, 44-52.

Clark, M.R., Johnson, T.J., Mccabe, R.T., Clark, J.T., Tuitupou, A., Elgendy, H., Friend, D.R., Kiser, P.F., 2012. A hot-melt extruded intravaginal ring for the sustained delivery of the antiretroviral microbicide UC781. J Pharm Sci 101, 576-587.

Dhumal, R.S., Kelly, A.L., York, P., Coates, P.D., Paradkar, A., 2010. Cocrystalization and simultaneous agglomeration using hot melt extrusion. Pharm Res 27, 2725-2733.

DiNunzio, J.C., Miller, D.A., Yang, W., McGinity, J.W., Williams III, R.O., 2008. Amorphous compositions using concentration enhancing polymers for improved bioavailability of itraconazole. Mol Pharm 5, 968-980.

Ewing, A.V., Biggart, G.D., Hale, C.R., Clarke, G.S., Kazarian, S.G., 2015. Comparison of pharmaceutical formulations: ATR-FTIR spectroscopic imaging to study drug-carrier interactions. Int J Pharm 495, 112-121.

Feng, X., Vo, A., Patil, H., Tiwari, R.V., Alshetaili, A.S., Pimparade, M.B., Repka, M.A., 2015. The effects of polymer carrier, hot melt extrusion process and downstream processing parameters on the moisture sorption properties of amorphous solid dispersions. J Pharm Pharmacol,2015.

Fule, R., Paithankar, V., Amin, P., 2016. Hot melt extrusion based solid solution approach: Exploring polymer comparison, physicochemical characterization and in-vivo evaluation. Int J Pharm 499, 280-294.

Furuyama, N., Hasegawa, S., Hamaura, T., Yada, S., Nakagami, H., Yonemochi, E., Terada, K., 2008. Evaluation of solid dispersions on a molecular level by the Raman mapping technique. Int J Pharm 361, 12-18.

Gryczke, A., Schminke, S., Maniruzzaman, M., Beck, J., Douroumis, D., 2011. Development and evaluation of orally disintegrating tablets (ODTs) containing Ibuprofen granules prepared by hot melt extrusion. Collo Sur B: Biointer 86, 275-284.

Irene, B., Veronica, A., Laura, A., Cosimo, C., 2014. A hyperbranched polyester as antinucleating agent for Artemisinin in electrospun nanofibers. Eur Polym J 60, 145-152.

Janssens, S., De Zeure, A., Paudel, A., Van Humbeeck, J., Rombaut, P., Van den Mooter, G., 2010. Influence of preparation methods on solid state supersaturation of amorphous solid dispersions: a case study with itraconazole and eudragit e100. Pharm Res 27, 775-785.

Kitak, T., Dumičić, A., Planinšek, O., Šibanc, R., Srčič, S., 2015. Determination of Solubility Parameters of Ibuprofen and Ibuprofen Lysinate. Molecules 20, 21549-21568.

Klein, C.E., Chiu, Y.-L., Awni, W., Zhu, T., Heuser, R.S., Doan, T., Breitenbach, J., Morris, J.B., Brun, S.C., Hanna, G.J., 2007. The tablet formulation of lopinavir/ritonavir provides similar bioavailability to the soft-gelatin capsule formulation with less pharmacokinetic variability and diminished food effect. JAIDS JAIDS J Acq Immu Def Syndr 44, 401-410.

Lauer, M.E., Siam, M., Tardio, J., Page, S., Kindt, J.H., Grassmann, O., 2013. Rapid assessment of homogeneity and stability of amorphous solid dispersions by atomic force microscopy—from bench to batch. Pharm Res 30, 2010-2022.

562 Lee, S.S., Hughes, P., Ross, A.D., Robinson, M.R., 2010. Biodegradable implants for sustained drug
563 release in the eye. Pharm Res 27, 2043-2053.
564 Liu, X., Lu, M., Guo, Z., Huang, L., Feng, X., Wu, C., 2012. Improving the chemical stability of
565 amorphous solid dispersion with cocrystal technique by hot melt extrusion. Pharmaceutical research 29,
566 806-817.
567 Loxley, A., 2013. Devices and Implants Prepared Using Hot Melt Extrusion, Melt Extrusion. Springer,
568 pp. 281-298.
569 Lu, J., Cuellar, K., Hammer, N.I., Jo, S., Gryczke, A., Kolter, K., Langley, N., Repka, M.A., 2015.
570 Solid-state characterization of Felodipine–Soluplus amorphous solid dispersions. Drug Development
571 and industrial pharmacy, 1-12.
572 Maniruzzaman, M., Boateng, J.S., Chowdhry, B.Z., Snowden, M.J., Douroumis, D., 2014. A review on
573 the taste masking of bitter APIs: hot-melt extrusion (HME) evaluation. Drug Dev Ind Pharm 40, 145-
574 156.
575 Marsac, P.J., Konno, H., Taylor, L.S., 2006. A comparison of the physical stability of amorphous
576 felodipine and nifedipine systems. Pharm Res 23, 2306-2316.
577 Maurin, M.B., Rowe, S.M., Blom, K., Pierce, M.E., 2002. Kinetics and mechanism of hydrolysis of
578 efavirenz. Pharm Res 19, 517-521.
579 Mishra, S., Tandon, P., Ayala, A., 2012. Study on the structure and vibrational spectra of efavirenz
580 conformers using DFT: Comparison to experimental data. Spectrochimica Acta Part A: Mol Biomol
581 Spectro 88, 116-123.
582 Moradiya, H., Islam, M.T., Woollam, G.R., Slipper, I.J., Halsey, S., Snowden, M.J., Douroumis, D.,
583 2013. Continuous cocrystallization for dissolution rate optimization of a poorly water-soluble drug.
584 Cryst Grow Des 14, 189-198.
585 Moradiya, H.G., Islam, M.T., Halsey, S., Maniruzzaman, M., Chowdhry, B.Z., Snowden, M.J.,
586 Douroumis, D., 2014. Continuous cocrystallisation of carbamazepine and trans-cinnamic acid via melt
587 extrusion processing. CrystEngComm 16, 3573-3583.
588 Moradiya, H.G., Nokhodchi, A., Bradley, M.S., Farnish, R., Douroumis, D., 2015. Increased dissolution
589 rates of carbamazepine-gluconolactone binary blends processed by hot melt extrusion. Pharm Dev
590 Tech, 1-8.
591 Palem, C.R., Kumar Battu, S., Maddineni, S., Gannu, R., Repka, M.A., Yamsani, M.R., 2013. Oral
592 transmucosal delivery of domperidone from immediate release films produced via hot-melt extrusion
593 technology. Pharm Dev Tech 18, 186-195.
594 Park, J.-B., Prodduturi, S., Morott, J., Kulkarni, V.I., Jacob, M.R., Khan, S.I., Stodghill, S.P., Repka,
595 M.A., 2015. Development of an antifungal denture adhesive film for oral candidiasis utilizing hot melt
596 extrusion technology. Exp Opin Drug Del 12, 1-13.
597 Pawar, J., Tayade, A., Gangurde, A., Moravkar, K., Amin, P., 2016a. Solubility and dissolution
598 enhancement of efavirenz hot melt extruded amorphous solid dispersions using combination of
599 polymeric blends: A QbD approach. Eur J Pharm Sci 88, 37-49.
600 Pawar, J.N., Shete, R.T., Gangurde, A.B., Moravkar, K.K., Javeer, S.D., Jaiswar, D.R., Amin, P.D.,
601 2015. Development of amorphous dispersions of artemether with hydrophilic polymers via spray
602 drying: Physicochemical and in silico studies. Asi J Pharm Sci,2015.
603 Pimparade, M.B., Morott, J.T., Park, J.-B., Kulkarni, V.I., Majumdar, S., Murthy, S., Lian, Z., Pinto,
604 E., Bi, V., Durig, T., 2015. Development of taste masked caffeine citrate formulations utilizing hot melt
605 extrusion technology and in vitro–in vivo evaluations. Int J Pharm 487, 167-176.
606 Repka, M.A., Majumdar, S., Kumar Battu, S., Srirangam, R., Upadhye, S.B., 2008. Applications of hot-
607 melt extrusion for drug delivery. Exp Opi Drug Del 5, 1357-1376.
608 Rowe, R.C., Sheskey, P.J., Quinn, M.E., 2009. Handbook of pharmaceutical excipients. Pharmaceutical
609 press.
610 Saerens, L., Dierickx, L., Lenain, B., Vervaet, C., Remon, J.P., De Beer, T., 2011. Raman spectroscopy
611 for the in-line polymer–drug quantification and solid state characterization during a pharmaceutical hot-
612 melt extrusion process. Eur J Pharm Biopharm 77, 158-163.
613 Sathigari, S.K., Radhakrishnan, V.K., Davis, V.A., Parsons, D.L., Babu, R.J., 2012. Amorphous-state
614 characterization of efavirenz—polymer hot-melt extrusion systems for dissolution enhancement. J
615 Pharm Sci 101, 3456-3464.

Sherman, E.M., Steinberg, J.G., 2011. Heat-stable ritonavir tablets: a new formulation of a pharmacokinetic enhancer for HIV. Exp Opi Pharmacoth 12, 141-148.
Stanković, M., de Waard, H., Steendam, R., Hiemstra, C., Zuidema, J., Frijlink, H.W., Hinrichs, W.L., 2013. Low temperature extruded implants based on novel hydrophilic multiblock copolymer for long-term protein delivery. Eur J Pharma Sci 49, 578-587.
Stanković, M., Frijlink, H.W., Hinrichs, W.L., 2015. Polymeric formulations for drug release prepared by hot melt extrusion: application and characterization. Drug Discov Today,2015.
Thommes, M., Ely, D.R., Carvajal, M.T., Pinal, R., 2011. Improvement of the dissolution rate of poorly soluble drugs by solid crystal suspensions. Mol Pharm 8, 727-735.
Turner, Y.T.A., Roberts, C.J., Davies, M.C., 2007. Scanning probe microscopy in the field of drug delivery. Adv Drug Del Rev 59, 1453-1473.
Urbanetz, N.A., 2006. Stabilization of solid dispersions of nimodipine and polyethylene glycol 2000. Eur J Pharm Sci 28, 67-76.
Van Krevelen, D.W., Te Nijenhuis, K., 2009. Properties of polymers: their correlation with chemical structure; their numerical estimation and prediction from additive group contributions. Elsevier.
Verma, G., Dhoke, S., Khanna, A., 2012. Polyester based-siloxane modified waterborne anticorrosive hydrophobic coating on copper. Sur Coat Tech 212, 101-108.
Vo, A.Q., Feng, X., Morott, J.T., Pimparade, M.B., Tiwari, R.V., Zhang, F., Repka, M.A., 2016. A novel floating controlled release drug delivery system prepared by hot-melt extrusion. Eur J Pharm Biopharm 98, 108-121.
Widjaja, E., Kanaujia, P., Lau, G., Ng, W.K., Garland, M., Saal, C., Hanefeld, A., Fischbach, M., Maio, M., Tan, R.B., 2011. Detection of trace crystallinity in an amorphous system using Raman microscopy and chemometric analysis. Euro J Pharm Sci 42, 45-54.
Yang, M., Wang, P., Suwardie, H., Gogos, C., 2011. Determination of acetaminophen's solubility in poly (ethylene oxide) by rheological, thermal and microscopic methods. Int J Pharm 403, 83-89.
Yeung, C.-W., Rein, H., 2015. Hot-melt extrusion of sugar-starch-pellets. Int J Pharm 493, 390-403.
Yu, L., Mishra, D.S., Rigsbee, D.R., 1998. Determination of the glass properties of D-mannitol using sorbitol as an impurity. J Pharm Sci 87, 774-777.
Zakeri-Milani, P., Barzegar-Jalali, M., Azimi, M., Valizadeh, H., 2009. Biopharmaceutical classification of drugs using intrinsic dissolution rate (IDR) and rat intestinal permeability. Eur J Pharm Biopharm 73, 102-106.
Zhang, Y., Wang, J., Bai, X., Jiang, T., Zhang, Q., Wang, S., 2012. Mesoporous silica nanoparticles for increasing the oral bioavailability and permeation of poorly water soluble drugs. Mol Pharm 9, 505-513.